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# How Do Neurons Choose Partners for Communication?

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In complicated interactive systems, and this includes the nervous system, choosing partners is one of the most difficult tasks. Since I do not speak your beautiful language I shall continue my lecture in the language which has most often served in partner-links between researchers over the last few decades.

Bonyolult interaktív rendszerekben, így az idegrendszerben is, a partnerválasztás a legnehezebb feladatok közé tartozik. Mivel nem beszélem az Önök szép nyelvét, előadásomat azon a nyelven folytatom, mely az utóbbi évtizedekben a kutatók közötti partnerkapcsolatokat leginkább szolgálta. — Let me therefore switch to English.

In complex interactive systems, even more than selecting a suitable language, it is difficult to choose appropriate partners for communication. The following example may elucidate the type of difficulty which arises: A fruitful partnership has been established between the Albert Szent-Györgyi University and the University of Göttingen. We would probably run into serious problems, if we were asked to explain the success of this partnership merely by evaluating the overall structures and functions of the two institutions involved. On the other hand, it is obvious that much of this success is based on cooperation between individual scientists and laboratories. Thus, the functional significance of local interactions apparently increases, when they fit the needs of both the whole system and the interacting components.

Similarly, neurobiological research has provided much evidence indicating that brain functions are closely related to interactions between specific sets of nerve cells. The functional development of the brain should then critically depend on how nerve cells choose partners for communication. This question includes three suppositions: nerve cells are capable of communication; nerve cells have specific communication partners; and nerve cells participate in the choice of their partners. I shall briefly discuss the validity of these assumptions, before we shall try to find an answer to the key question: Which criteria do nerve cells use for selecting communication partners?

The first assumption, *nerve cells are capable of communication,* is based on characteristic properties of nerve cells or neurons. These may be summarized as follows:

— Nerve cells release and receive signals

— Signal transfer between neurons by "chemical transmitters" is directed through cell-to-cell contacts ("synapses") (Fig. 1).

— Nerve cells are polarized between transmitting and receiving modules which spatially separate the "pre- and post-synaptic elements" of each neuron.

— Two types of post-synaptic effect ("excitation" and "inhibition") are mediated by separate neuron populations.

— Synaptic messages regulate the probability of signal formation by disturbing the excitation-inhibition balance in the receiving neuron.

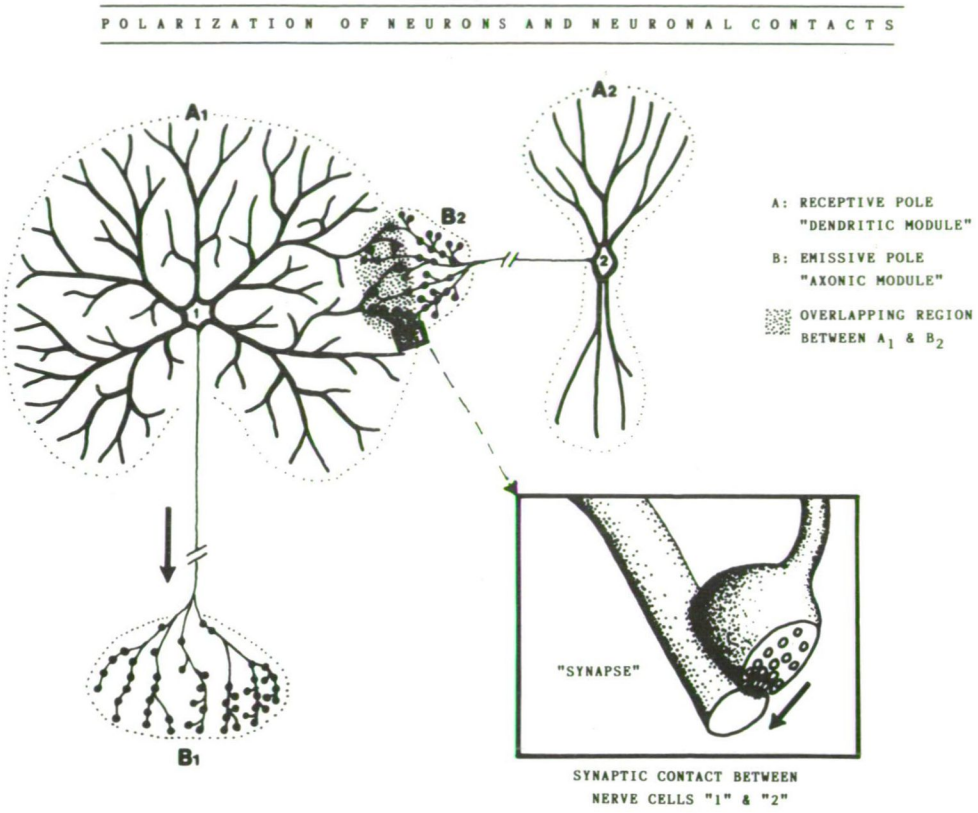


*Fig. 1:* Neurons are polarized cells. Presynaptic and postsynaptic elements spatially separate and accumulate (axons and dendrites, respectively). "Synapses" are contacts between pre- and postsynaptic elements which are specialized for directed signal transfer by chemical transmitters. Synaptic connections between neurons are restricted to the overlapping region between their "receptive" and "emissive" poles.

Thus, nerve cells indeed show cytological characteristics which make them suitable for intercellular communication. Apparently they form a cable-communication system with plug-and-socket-like contacts through which they may exert excitatory or inhibitory effects on each other.

The second question is, whether or not *nerve cells have specific communication partners*. There is an enormous complexity of intercellular connections in the nervous system and many of these connections may be neither stable nor specific. However, physiological, chemical and anatomical data suggest that neurons may have highly specific connections which characterize their function. We then have to ask: How specific are connections between neurons?

— Between all neurons, connections are restricted by the range of their cell processes (Fig. 2).

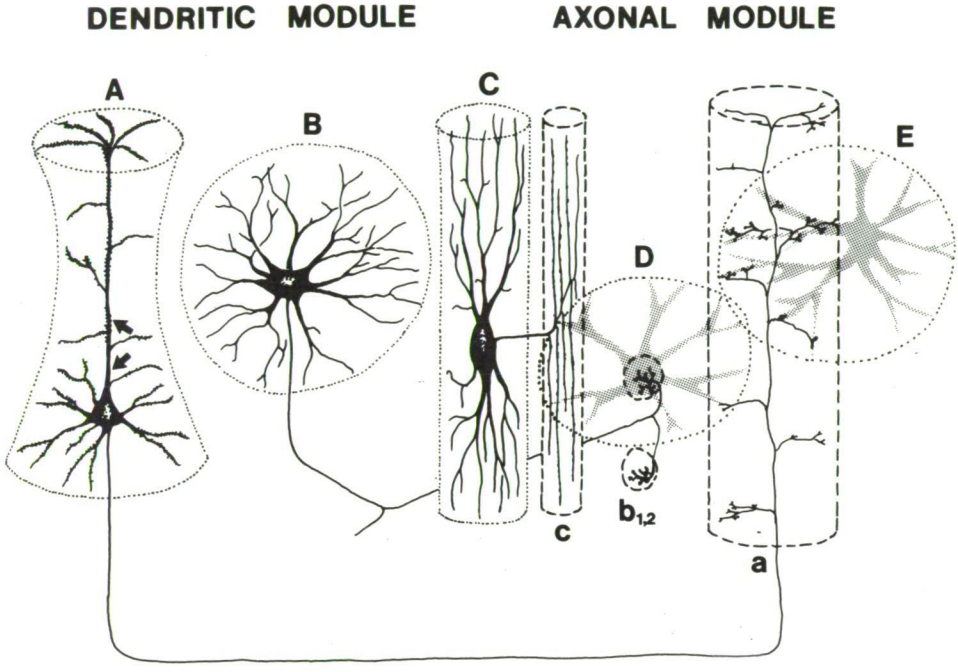


*Fig. 2:* Neurons can be classified according to the size, shape and position of their "dendritic" and/or "axonal modules". Projection neurons (A) have long axons and make synaptic contacts to target cells in remote positions (synapses of A and E). Local interneurons (B, C) have short axons and may focus their synapses on specific target cells, e.g. „basket cells" which effectively inhibit other neurons at their perikaryon (synapses of B on D).

— Statistically, each neuron is connected to an extremely small subpopulation of all neurons (mammalian brains contain $10^7$ to $10^{11}$ neurons; each neuron forms and receives $10^0$ to $10^4$ synapses; the ratio between connected and unconnected neurons is therefore probably smaller than 1:1 million).

— Neuron classes can be defined by the size and shape of their "receiving cables" and/or "transmitting cables" (see Figs. 1 and 2).

— Projection neurons have long axons establishing synaptic contacts to specific target cells in remote positions (neuron A in Fig. 2).

— Local interneurons have short axons selectively innervating specific neighbouring cells but avoiding others (e.g. "basket cells", neuron B in Fig. 2).

— There are often countercurrent connections between neurons in different parts of the nervous system (see Fig. 3).

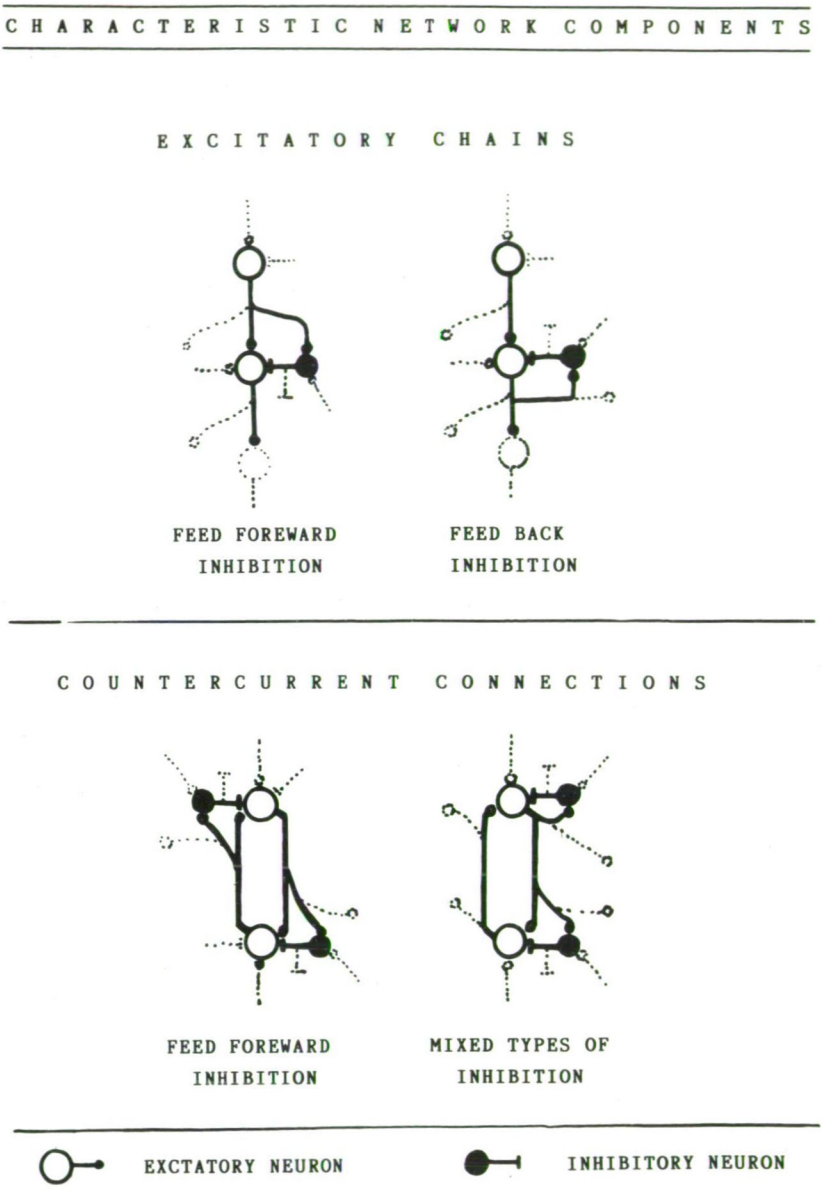


*Fig. 3:* Characteristic constellations of excitatory and inhibitory neurons which can be isolated from complex networks in the nervous systems. Note the characteristic involvement of various types of feed-forward- or feedback-inhibition. Dotted lines represent disregarded connections.

Thus, neuronal connections are selective, i.e. they are restricted to extremely small subpopulations of all neurons. Some of them are apparently specific, because they are focused on specific partner cells.

The third suggestion is that *neurons participate in the choice of their communication partners.* This assumption is supported by experimental evidence. During ontogenesis, synaptic connections primarily show a relatively diffuse distribution but are secondarily specified. This remodelling is often based on selective elimination of synapses (Fig. 4.). On the other hand, selective sprouting of axons is observed when synaptic connections are lost (e.g. after lesions; Fig. 5). Reactive synaptogenesis reveals that a hierarchy exists in the specificity of neuronal connections. Thus, neurons are apparently involved in the choice of communication partners. The choice may be based on selective formation and/or elimination of synaptic connections.

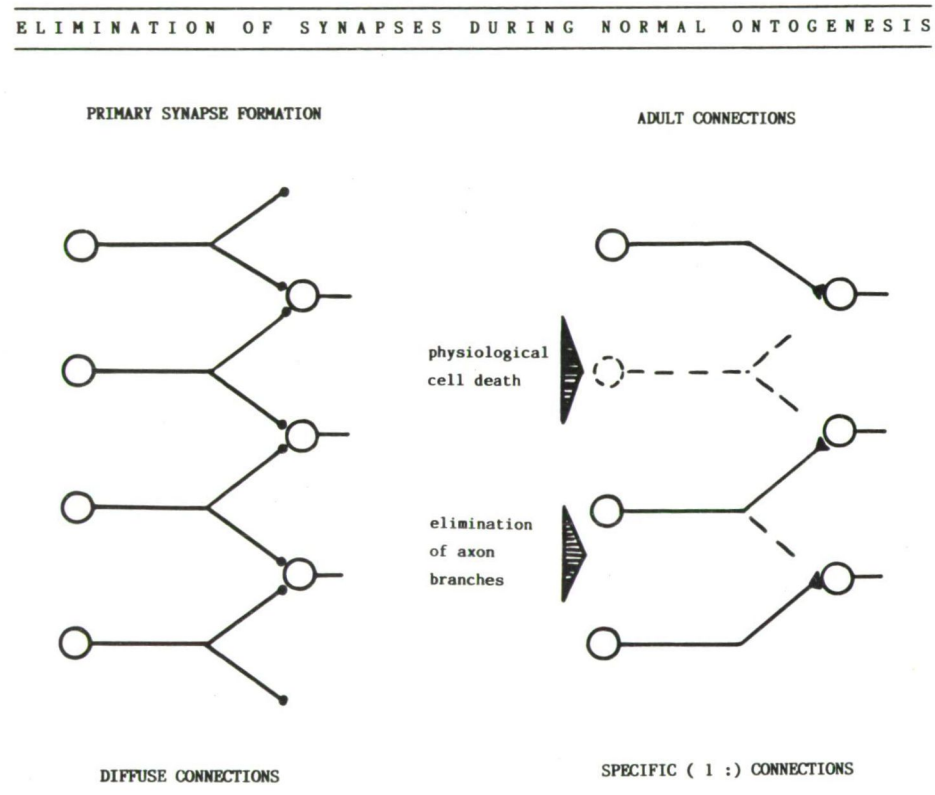


*Fig. 4:* During ontogenesis, many connections between sets of neurons are secondarily specified by elimination of inappropriate connections. This may be based on a loss of axon collaterals, but may also include cell death of significant fractions of neurons. Thus, brain development shows critical periods of synaptic reorganization. These may result in „catastrophies" on the level of cells or networks and apparently serve to stabilize the surviving connections.

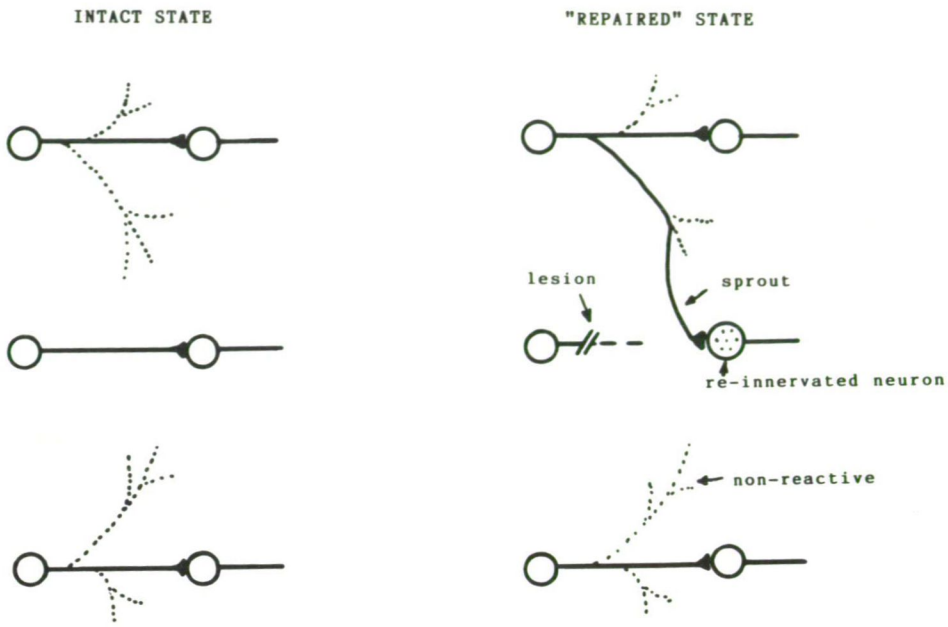


*Fig. 5:* In the adult nervous system, experimental lesions or pathological processes may induce synapse degeneration. This in turn results in synaptic reorganization, which may include selective sprouting of some axon types, while others do not react at all. Thus, re-innerveration is a selective process. Reactive synaptogenesis reveals a hierarchy of compensatory mechanisms which regulate the connectivity of the nervous system.

Now, we may ask the main question: *What are the conditions on which neurons form and stabilize connections to other neurons?* We have seen that each synapse consists of pre- and postsynaptic elements. In certain conditions, however, one can observe supernumerous ("free" or "vacant") presynaptic or postsynaptic elements which did not find appropriate contact partners. This finding indicates that pre-and postsynaptic elements may be formed or eliminated independently from each other. Therefore, the stabilization of a synapse seems to depend on proper conditions in both the presynaptic neuron and the postsynaptic neuron. We then have to specify our question by asking: What are the conditions in which neurons form and maintain presynaptic or postsynaptic elements? And are these conditions different for excitatory and inhibitory synapses?

In vivo and vitro experiments suggest that increased excitation of neurons stimulates the *formation of presynaptic elements.* In addition, there is indirect evidence that loss of inhibition may have a similar effect as increased excitation. On the other hand, increased inhibition and a loss of excitation may reduce the growth of axons and, therefore, reduce the number of presynaptic elements. At present, there is no evidence that excitatory and inhibitory neurons react in a different manner (Fig. 6).

| RESPONDING SYNAPSE COMPONENT | | CHANGES IN INPUT E↑ | I↓ | E↑ | I↓ |
|---|---|---|---|---|---|
| presynaptic elements | E | + | (+) | − | − |
| | I | + | (+) | − | − |
| postsynaptic elements | E | − | (−) | + | + |
| | I | (+) | (+) | − | − |
| | | E/I ↑ | | E/I ↓ | |

On the other hand, are there conditions that promote the *formation of postsynaptic elements?* We know very little about the dynamics of postsynaptic elements of *inhibitory* synapses. These synapses tend to accumulate on perikarya and on proximal dendrites rather than on peripheral ones. This distribution pattern indicates that inhibitory postsynaptic elements increase in number where excitatory postsynaptic potentials converge and summate. On the other hand, there are reports on hypersensitivity for inhibitory transmitters developing as a consequence of decresased or lost inhibitory input. These have been tentatively summarized as shown in the last row of Fig. 6.

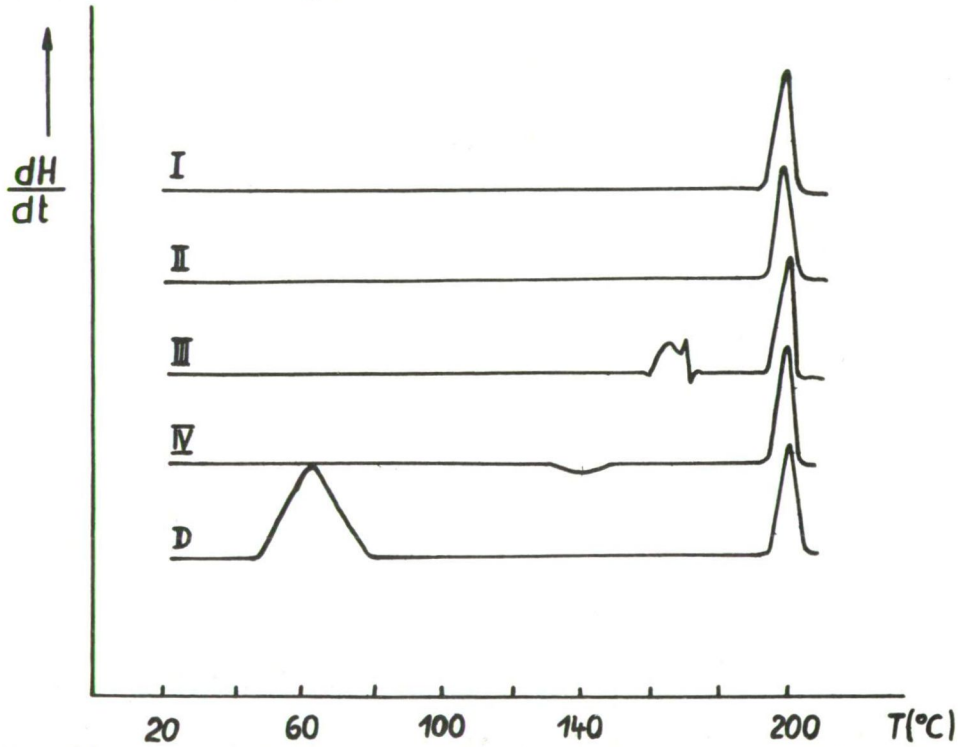


*Fig. 6:* Scheme of synaptic plasticity reactions following longterm disturbances of the excitation/inhibition balance (E/I). These reactions may be induced by changes in input (E ↑, ↓; I ↑, ↓) but also by other factors such as certain hormones, growth promoting factors, etc. (not included). "Plus": increase in number or efficacy of pre- or postsynaptic elements, i. e. their formation is promoted and/or their breakdown is diminished. "Minus": decrease in number or efficacy of synaptic elements, formation retarded and/or breakdown increased. Brackets indicate that direct experimental evidence is not yet available, but circumstantial evidence exists (for details see Wolff und Wagner, 1983; Dammasch et al. 1986; Wagner and Wolff, 1988).

Vacant *postsynapic densities of excitatory* synapses transiently occur in various parts of the nervous system. Experimentally, their appearance can be induced by lesions which destroy excitatory axon systems. Thus, when the excitatory input decreases vacant postsynaptic elements for making excitatory synapses appear (Fig. 6). Since inhibition can decrease the efficacy of excitatory input in postsynaptic neurons, one may wonder whether increased inhibition affects the number of "vacant" (excitatory) postsynaptic elements in a similar manner as reduced excitation.

At this point, I shall briefly summarize some results which were obtained in a long lasting cooperation with colleagues from Szeged (listed below).

Co-workers in Szeged

| | |
|---|---|
| JOO, F.<br>HALÁSZ, N.<br>PÁRDUCZ, Á.<br>SIKLÓS, L. | Biological Research Center<br>(Molecular Neurobiology Unit) |
| KÁSA, P.<br>DOBÓ, E.<br>GULYA, K.<br>RAKONCZAI, Z. | Szent-Györgyi Albert Medical University<br>(Central Research Laboratory) |
| FEHÉR, O.<br>TÓLDI, J.<br>ROJIK, I. | Attila József University<br>(Dept. of Comparative Physiology) |

Co-workers in Göttingen

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| BALCAR, V. J.<br>DAMES, W.<br>DAMMASCH, I.<br>HOLZGRAEFE, M.<br>LEUTGEB, U.<br>MICHLER, A.<br>RICKMANN, M.<br>WAGNER, G. | Georg August University<br>(Dept. of Anatomy and Neurology) |

For most of these multidisciplinary studies, we selected the superior cervical ganglion. In rats, this ganglion contains a relatively small set of neurons which receive excitatory input via acetylcholinergic axons from the spinal cord. The principal ganglion cells provide the sympathetic (noradrenergic) innervation to the head region. It was demonstrated that, apart from other interneurons, this ganglion includes GABAergic components. These are driven by preganglionic input and inhibit the principal ganglion cells (Fig. 7A, Wolff et al., 186; Kása et al., 1988). The

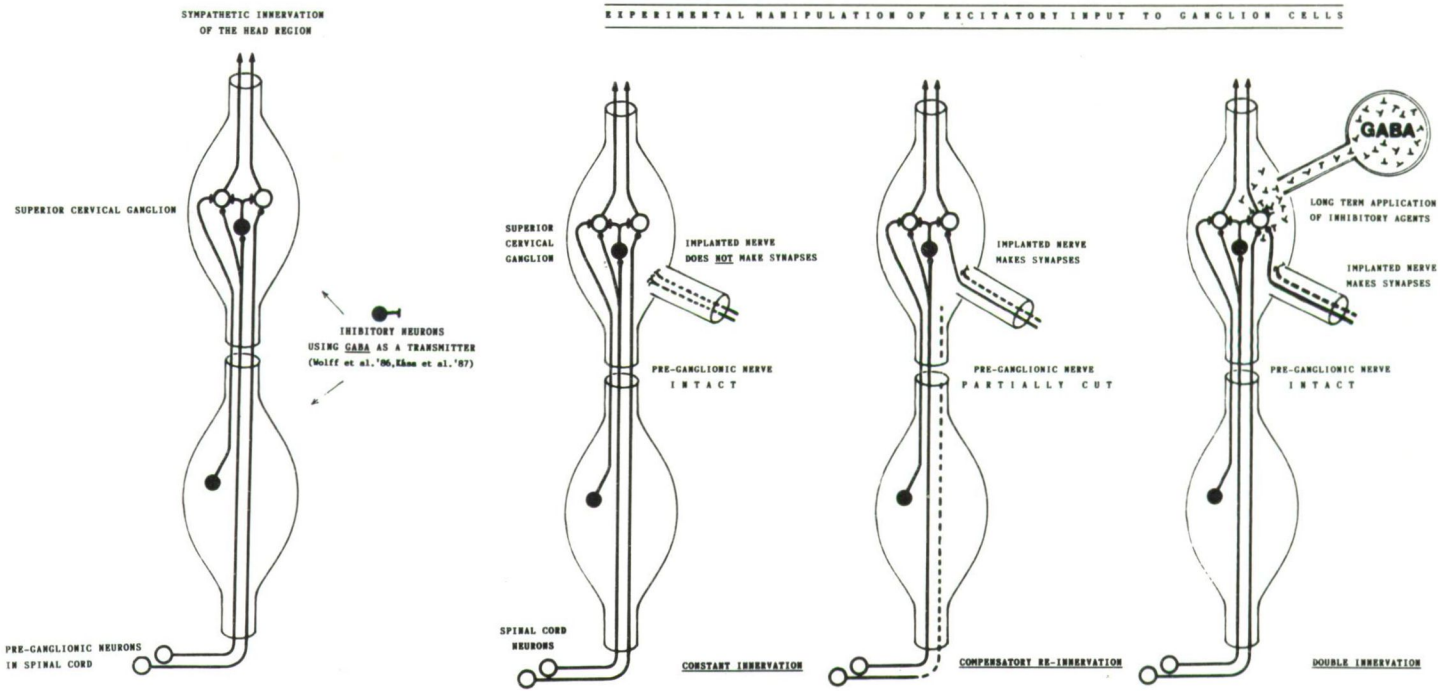


*Fig. 7:* Scheme of synaptic components and neuroplastic reactions in the superior cervical ganglion of rats. *A:* normal ganglion (SIF cells and other interneurons are not shown); *B:* foreign nerve implanted into the normal ganglion is not able to make functional synapses; *C:* after partial de-afferentation (interruption of preganglionic axons) implanted acetylcholinergic nerves do make functional synapses on principal ganglion cells; *D:* a surplus of inhibition induced by exogenous GABA provides conditions in which implanted nerves do make synapses in the presence of intact preganglionic nerves.

existence of an endogenous GABA-system may explain why externally applied GABA (gamma- aminobutyric acid) strongly inhibits the activation of ganglion cells when the preganglionic nerve trunk is stimulated (Farkas et al., 1986).

The superior cervical ganglion was a suitable model for our experiments, (review see Wolff et al., 1987) because it had been previously demonstrated that the hypoglossal or vagal nerve can be implanted in vivo into this ganglion. We confirmed that the foreign nerve does *not* make synapses in this ganglion as long as the preganglionic nerve is intact (Fig. 7B). We also confirmed that the implanted nerve does, however, make functional (acetylcholinergic) synapses on ganglion cells when the preganglionic nerve had previously been cut, at least partially (Fig. 7C). Interestingly enough, long-term application of exogenous GABA induced the formation of vacant postsynaptic densities for excitatory synapses. In addition, the number of acetylcholin-receptors transiently increased ("hypersensitivity), if GABA was infused into ganglia with an intact preganglionic nerve. In other words, if none of the above-mentioned nerves was implanted in addition to the GABA-applicator, the nerve was able to make functional synapses on ganglion cells, although the preganglionic nerve was intact (Fig. 7D). This indicates that both decrease of excitatory input and hyperinhibition have a similar effect on the acceptance of excitatory synapses (Fig. 6).

In both cases, the formation of synapses depends on additional (acetylcholinergic) presynaptic elements provided by the implanted (regenerating) nerve. Viewed from the level of the organism, the „double innervation" of a sympathetic ganglion by a preganglionic sympathetic nerve and a motor nerve (hypoglossal n.) or a parasympathetic nerve (vagal n.) does not make sense. At the cellular level, however, ganglion cells may overcome hyperinhibtion by additional excitatory input. This indicates that the amount of pre- and/or postsynaptic elements formed and stabilized by neurons depends on local conditions. If these conditions change, neurons will show plastic reactions and the synaptic connections will be reorganized, if appropriate partners can be found.

This "trophic" effect of hyperinhibition is not specific for GABA. Similar plastic responses may be induced by long-term application of sodium bromide (NaBr, „tranquillizer of the 19th century") which does not require local application, but is taken orally via the drinking water in therapeutic-like doses (140—280 $\mu$g/l). Thus, synaptic plasticity can also be induced by long-term pharmacotherapy of drugs which increase neural inhibition.

In normal conditions, inhibition and excitation of neurons mainly depends on synaptic input (and in some cases on hormonal effects). Synaptic input is regulated by transneuronal interactions. In Fig. 8A–C it is demonstrated how such transsynaptic effects might influence the formation and stabilization of synapses. Probably, such mechanisms are also involved in normal synapse formation, because characteristic combinations of excitatory and inhibitory neurons are found in the brain and spinal cord (Fig. 3).

We may *summarize* this view of how neurons choose communication partners, as follows:

— The choice of communication partners is based on selective stabilization and breakdown of synapses.

— In each neuron, the balance between excitation and inhibition plays a role in the stabilization or elimination of synapses.

— A neuron may distinguish between "good" and "bad" synapses depending on whether they stabilize or disturb the balance.

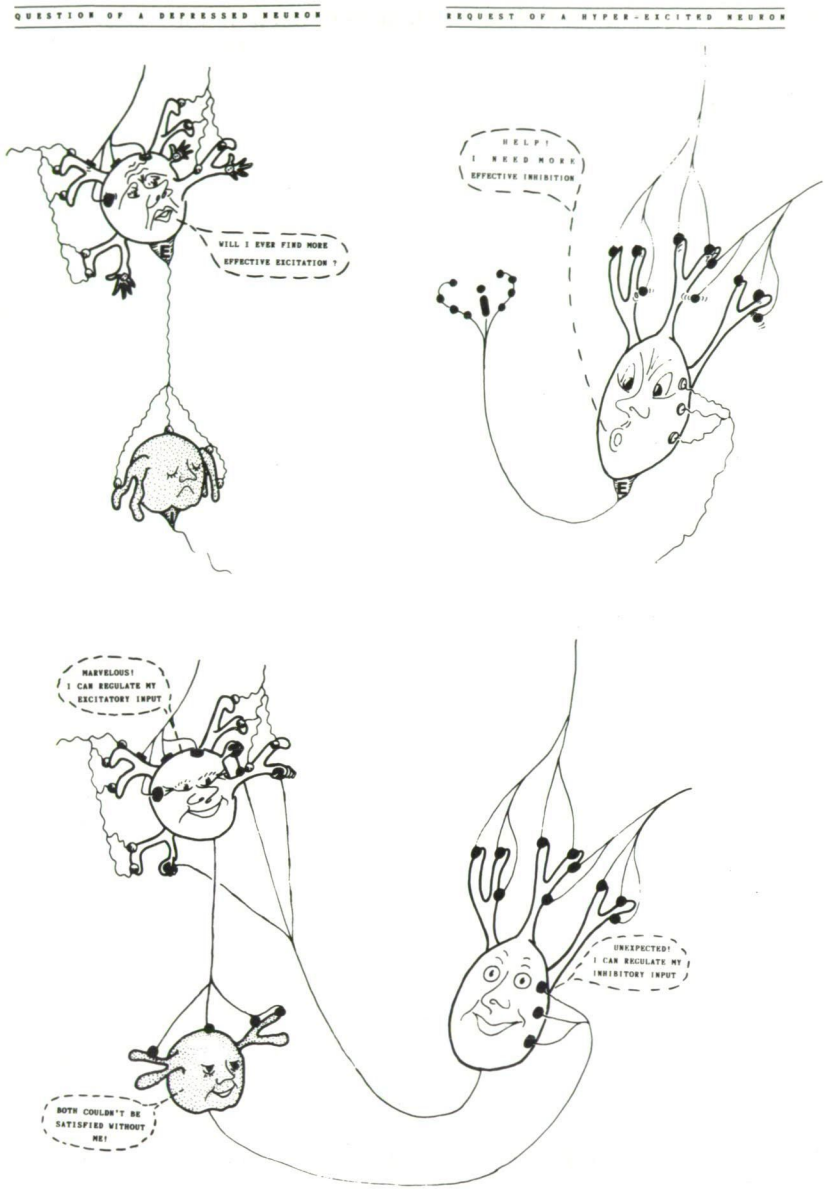


*Fig. 8:* Cartoon-like illustration of how neurons may cooperate in the formation and stabilizion of synaptic connections within small networks.

— In this way, each neuron may choose communication partners because of their local effects.

— Since synaptic effects depend on cell-to-cell interactions in neuronal networks, synapse formation can compensate disbalanced states of neuronal networks, and vice versa.

— In this way, synaptic connections may be adapted to the requirements of the nervous system and may be modified by sensory input, although their formation and maintenance is based on local decisions.

— We may conclude that the choice of communication partners in the nervous system is itself a result of communication between neurons and neuronal networks.

*Implications and applications of this concept*

It is proposed that synaptic connections are formed, maintained or eliminated according to rules which tend to compensate disturbances of the excitation-inhibition balance of the neurons concerned (see Fig. 6). The rules are based on extreme simplifications and their experimental basis is still imcomplete. The heuristic value of such a working hypothesis obviously depends on whether or not it leads to new approaches to the nervous system. Here, I shall focus on four aspects of neurobiology:

*Basic research:* It is difficult to study interaction mechanisms in complex systems. One of the major obstacles is that experiments require identical reproduction which can hardly be achieved in complex interactive systems. One way to overcome this restriction is the formalization of theories followed by computer simulation. Biological experiments may then be restricted to testing critical predictions from "simulated experiments". This can be done with the "compensation theory of synaptogenesis" (see Wolff and Wagner, 1983; Dammasch et al., 1986).

*Ontogenesis:* It is one of the challenges of developmental neurobiology to explain the interaction between genetic information and epigenetic mechanisms. The concept predicts that brain development is regulated by at least three types of factors: (1) Trophic factors comprise all agents and conditions (including synaptic input) which affect any part of the molecular cascade which leads to formation or breakdown of pre- or postsynaptic elements and/or synaptic contacts; (2) spatial and temporal maturation patterns of excititatory and inhibitory neurons; (3) environmental factors include not only information transfer from sensory organs and noxious influences on the formation or stabilization of synapses.

*Pathology:* The concept claims that the stability of connections in the normal brain results from a dynamic equilibrium of productive and degrading mechanisms. Following lesions, we will have to identify different conditions in which synaptic reorganization either results in functional rehabilitation or leads to progressive dysfunction (degenerative diseases).

*Pharmaco-therapy:* According to the compensation theory of synaptogenesis, we have to be aware of the possibility that synaptic connections may be modified by long-term application of drugs which interfere with synaptic transmission.

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